SEPARATION AND PURIFICATION OF POLY-3-HYDROXYALKANOIC ACID

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Abstract of **JP2001046094**

PROBLEM TO BE SOLVED: To separate and purify the subject compound which is a biodegradable plastic by adding a surfactant to a suspension of a microbial cell of a microorganism containing a poly-3-hydroxyalkanoic acid and carrying out a physical crushing treatment of the resultant mixture liquid. SOLUTION: A surfactant is added to a suspension of a microbial cell of a microorganism (e.g. Aeromonas caviae) containing a poly-3-hydroxyalkanoic acid comprising a bipolymer of D-3-hydroxybutyrate and D-3-hydroxyhexanoate, a terpolymer, etc., of the D-3-hydroxybutyrate, D-3-hydroxyvalerate and D-3- hydroxyhexanoate and the resultant mixture liquid is then subjected to a physical crushing treatment to thereby separate and purify the objective poly-3- hydroxyalkanoic acid suitable as a raw material, etc. for a plastic product, an implant material without requiring recovery, a drug carrier, a fertilizer carrier, an agricultural mulching film, a fishing gear such as a fishing line, a bag, etc. such as a refuse bag, etc. for composts in high purity and high yield.

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(54)【発明の名称】　ポリ－３－ヒドロキシアルカン酸の分離精製方法

(57)【要約】
【課題】ＰＨＡを含有する微生物菌体から、少ない工程数で高純度のＰＨＡを高収率で得ることのできるＰＨＡの分離精製方法を提供すること。
【解決手段】ポリ－３－ヒドロキシアルカン酸を含有する微生物菌体の懸濁液に界面活性剤を添加し、得られる混合液を物理的破砕処理することを特徴とするポリ－３－ヒドロキシアルカン酸の分離精製方法。

れない。例えば、アルカリゲネス・リポリチカ（Ａｌｃａｌｉｇｅｎｅｓ　ｌｉｐｏｌｙｔｉｃａ）、アルカリゲネス・ユウトロファス（Ａ．ｅｕｔｒｏｐｈｕｓ）、アルカリゲネス・ラタス（Ａ．ｌａｔａｓ）等のアルカリゲネス属（Ａｌｃａｌｉｇｅｎｅｓ）、シュウドモナス属（Ｐｓｅｕｄｏｍｏｎａｓ）、バチルス属（Ｂａｃｉｌｌｕｓ）、アゾトバクター属（Ａｚｏｔｏｂａｃｔｅｒ）、ノカルディア属（Ｎｏｃａｒｄｉａ）、アエロモナス属（Ａｅｒｏｍｏｎａｓ）の菌が挙げられ、中でも、アエロモナス・キャビエ（Ａｅｒｏｍｏｎａｓ　ｃａｖｉａｅ）等の菌株、またはアエロモナス・キャビエ由来のＰＨＡ合成酵素群の遺伝子が導入された菌株、例えば、アルカリゲネス・ユウトロファスＡＣ３２（寄託番号ＦＥＲＭ　Ｐ－１５７８６）（Ｊ．Ｂａｃｔｅｒｉｏｌ．，１７９，４８２１－４８３０頁（１９９７））等があり好ましい。

【００１０】これらの微生物の培養方法は、ＰＨＡを多量に効率よく菌体内に蓄積できるものであれば特に限定はなく、例えば、前記アルカリゲネス・ユウトロファスＡＣ３２（ＦＥＲＭ　Ｐ－１５７８６）を用いる場合には、Ｊ．Ｂａｃｔｅｒｉｏｌ．，１７９，４８２１－４８３０頁（１９９７）等に記載の方法が好ましい。

【００１１】本発明におけるポリ－３－ヒドロキシアルカン酸（ＰＨＡ）とは、Ｄ－３－ヒドロキシブチレート（３ＨＢ）のホモポリマーや３ＨＢと他のＳ－ヒドロキシアルカン酸との共重合体などを示すが、中でも３ＨＢとＤ－３－ヒドロキシヘキサノエート（３ＨＨ）との２成分共重合体（Ｍａｃｒｏｍｏｌｅｃｕｌｅｓ，２８，４８２２－４８２８（１９９５））または３ＨＢとＤ－３－ヒドロキシバレレート（３ＨＶ）と３ＨＨとの３成分共重合体（特開平８－２８９７９７号公報）が、物性の面からより好ましい。ここで３ＨＢと３ＨＨの２成分共重合体を構成する各モノマーユニットの組成比については、特に限定されるものではないが、３ＨＢの含有量が１～９９モル％、３ＨＨユニットの含有量が１～９９モル％のものが好適である。また、３ＨＢと３ＨＶと３ＨＨとの３成分共重合体を構成する各モノマーユニットの組成比については特に限定されるものではないが、例えば、３ＨＢユニットの含有量が１～９５モル％、３ＨＶユニットの含有量が１～９６モル％、３ＨＨユニットの含有量が１～３０モル％のものが好適である。また、これらのＰＨＡの分子量は、１０万以上が好ましく、５０万以上がより好ましい。

【００１２】ＰＨＡの微生物菌体中の含有率は、高い方が好ましいのは当然であり、工業レベルでの適用においては乾燥菌体中に２０重量％以上が好ましく、界面活性剤処理、物理的破砕処理、分離操作、分離したＰＨＡの純度等を考慮すると５０重量％以上が特に好ましい。

【００１３】本発明においては、前記のように培養して得られた微生物菌体の懸濁液に界面活性剤を添加する。なお、本発明における「微生物菌体の懸濁液」とは、培養終了後の培養懸濁液または培養液から遠心分離等で分離した菌体を水に懸濁させた水性の懸濁液を意味する。該懸濁液中における菌体濃度は、湿菌体換算で５００ｇ／ｌ以下が好ましく、３００ｇ／ｌ以下がさらに好ましい。

【００１４】本発明で使用する界面活性剤としては、陰イオン性、陽イオン性、両性もしくは非イオン性でも良く、具体的には、ドデシル硫酸ナトリウム、ドデシルスルホン酸ナトリウム、コール酸ナトリウム、デオキシコール酸ナトリウム、オレイン酸ナトリウム、セチルトリメチルアンモニウムブロミド、ドデシルピリジニウムクロリド、３－（（３－コラミドプロピル）ジメチルアンモニオ）－１－プロパンスルホン酸、３－（（コラミドプロピル）ジメチルアンモニオ）－２－ヒドロキシ－１－プロパンスルホン酸、ドデシルＮ－ベタイン、オクチルグルコシド、ヘプチルチオグルコシド、ポリエチルエチレンドデシルエーテル、ポリオキシエチレンノニルフェニルエーテル、ポリオキシエチレンソルビトールエステル等が挙げられるが、これらに制限されるものではない。本発明においては、特にドデシル硫酸ナトリウム、ドデシルスルホン酸ナトリウム、コール酸ナトリウム、デオキシコール酸ナトリウム、オレイン酸ナトリウム等が、価格、使用量や添加効果の点から好ましい。

【００１５】界面活性剤の添加量は、特に制限されないが、微生物菌体重量（湿菌体換算）１００重量部に対して、０．００１～５０重量部が好ましく、１～２０重量部がより好ましい。該添加量は、界面活性剤の添加効果が良好な観点から、０．００１重量部以上が好ましく、低コストである観点から、５０重量部以下が好ましい。また、得られた混合液は、ＰＨＡ以外の菌体構成成分の可溶化を促進させる観点から、室温下で１分～２時間程度撹拌することが好ましい。

【００１６】次いで、前記混合液を物理的破砕処理する。本発明においては、かかる物理的破砕処理を行なうことにより、前記微生物菌体を破砕してＰＨＡを菌体外に漏出させる効果を有する。

【００１７】本発明における物理的破砕処理とは、超音波による破砕、高圧ホモジナイザーやミル等による破砕等が挙げられる。高圧ホモジナイザーとしては、独国のＡＰＶ・ゴーリン社製「マントンゴーリン（商品名）」、デンマークのＡＰＶラニー社製「ミニラボ（商品名）」、米国のマイクロフルイディックス（Ｍｉｃｒｏｆｌｕｉｄｉｃｓ）社製「マイクロフルイタイザー（商品名）」等が挙げられ、ミルとしては、スイスのウィリー・エー・バックオフェン（Ｗｉｌｌｙ　Ａ．Ｂａｃｈｏｆｅｎ）社製「ダイノーミル（商品名）」等が挙げられるが、同等の破砕効果が得られればこれらに限定されるものではない。

【００１８】物理的破砕処理の条件としては、用いる手段により一概には限定できないが、例えば、超音波による破砕の場合、米国のブランソン（Ｂｒａｎｓｏｎ）社製、ソニ

なわなかった以外は同様の操作を行なった。その結果、遠心分離しても沈殿物は得ることはできず、ポリマーは全く分離できなかった。

【００３１】比較例２

実施例１においてドデシル硫酸ナトリウム処理を行なわなかった以外は同様の操作を行なった。その結果、遠心分離して得られた沈殿物の純度は、懸濁前のポリ（３ＨＢ－ｃｏ－３ＨＨ）含有微生物菌体の純度と同じ５０％であった。

【００３２】比較例３

ポリ（３ＨＢ－ｃｏ－３ＨＨ）含有微生物菌体の懸濁液１００ｍｌを「ダイノーミル」を用いて１１／ｈの流速で１時間破断処理した後、１０ｇ／ｌになるようにドデシル硫酸ナトリウムを加えて室温で１時間攪拌した。得られた菌体懸濁液は非常に粘重で、遠心分離処理してもポリ（３ＨＢ－ｃｏ－３ＨＨ）を得ることはできなかった。

【００３３】以上の結果より、実施例１～３で得られた＊ポリ（３ＨＢ－ｃｏ－３ＨＨ）は、いずれも界面活性剤を使用していない比較例２で得られたものに比べ、高純度のものであることがわかる。

【００３４】また、実施例１～３及び比較例１～３の結果より、ポリ（３ＨＢ－ｃｏ－３ＨＨ）の分離精製方法には、物理的破砕処理と界面活性剤の添加の両方が必要であるが、界面活性剤の添加処理液を物理的破砕処理することにより顕著な効果が得られる。

【００３５】

【発明の効果】本発明によれば、高純度のポリ－３－ヒドロキシアルカン酸（ＰＨＡ）を効率よく、極めて簡便に得られるため、本発明は、ＰＨＡの工業的生産の効率向上およびコストの低減に大きく寄与するものである。また、本発明により得られるＰＨＡは、実用品として十分に高い純度を有するものであり、例えば、プラスチック製品、回収不要のインプラント材料、薬物担体、肥料担体、農業用マルチフィルム、釣糸等の漁具、コンポスト用ゴミ袋等の原料として好適に用いられる。

フロントページの続き

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(54) SEPARATION AND PURIFICATION OF POLY-3-HYDROXYALKANOIC ACID

(57)Abstract:
PROBLEM TO BE SOLVED: To separate and purify the subject compound which is a biodegradable plastic by adding a surfactant to a suspension of a microbial cell of a microorganism containing a poly-3-hydroxyalkanoic acid and carrying out a physical crushing treatment of the resultant mixture liquid.
SOLUTION: A surfactant is added to a suspension of a microbial cell of a microorganism (e.g. Aeromonas caviae) containing a poly-3-hydroxyalkanoic acid comprising a bipolymer of D-3-hydroxybutyrate and D-3-hydroxyhexanoate, a terpolymer, etc., of the D-3-hydroxybutyrate, D-3-hydroxyvalerate and D-3- hydroxyhexanoate and the resultant mixture liquid is then subjected to a physical crushing treatment to thereby separate and purify the objective poly-3-hydroxyalkanoic acid suitable as a raw material, etc. for a plastic product, an implant material without requiring recovery, a drug carrier, a fertilizer carrier, an agricultural mulching film, a fishing gear such as a fishing line, a bag, etc. such as a refuse bag, etc. for composts in high purity and high yield.

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CLAIMS

[Claim(s)]
[Claim 1] The separation purification approach of the Polly 3-hydroxy alkane acid characterized by adding a surfactant to the suspension of the microorganism biomass containing a Polly 3-hydroxy alkane acid, and carrying out physical crushing processing of the mixed liquor obtained.
[Claim 2] The separation purification approach according to claim 1 that a Polly 3-hydroxy alkane acid is 3 component copolymer of 2 component copolymer of D-3-hydroxy butyrate (3HB) and D-3-hydroxy hexanoate (3HH) or D-3-hydroxy butyrate (3HB), D-3-hydroxyvalerate (3HV), and D-3-hydroxy hexanoate (3HH).
[Claim 3] The separation purification approach according to claim 1 or 2 that the microorganism containing a Polly 3-hydroxy alkane acid is the strain into which the Polly 3-hydroxy alkane acid synthetic enzyme group gene of the Aeromonas KYABIE origin was introduced.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the separation purification approach from the microorganism biomass of a Polly 3-hydroxy alkane acid.
[0002]
[Description of the Prior Art] Although current and a plastic waste are processed by incineration, reclamation, etc., there are troubles, such as warming of the earth and ground relaxation of a reclaimed ground, in these arts. Therefore, recycle system-ization is progressing with a rise of the social consciousness to plastics recycle. However, as for the actual condition, there is much what remains there being a limitation in a recyclable application, could not respond only by incineration, reclamation, and recycle as a plastics abolition art actually, and left in a nature. Then, after abolition, it is incorporated by the cyclical change of materials of a nature, the biodegradable plastic from which a decomposition product does not become harmful attracts attention, and it is anxious for the utilization.
[0003] Also in these biodegradable plastics, a Polly 3-hydroxy alkane acid (PHA is called henceforth) is thermoplastic polyester which is generated and is accumulated as energy are recording matter into the biomass of many microorganism kinds, and since it is incorporated by the carbon cycle process of a nature, and it is expected that there is almost no adverse effect to an ecosystem, it attracts attention especially. Moreover, also in the medical field, it is thought that the implant material of recovery needlessness and the utilization as a drug carrier are possible.
[0004] Said PHA forms the microsome, is accumulated into the microorganism biomass, and in order to use these as plastics, it needs to carry out separation purification out of a microorganism biomass. As a known approach of carrying out separation purification of the PHA from a microorganism biomass, when it divides roughly, there are an approach of PHA dissolving PHA in a meltable organic solvent, and extracting PHA, and a method of obtaining PHA by making biomass constituents other than PHA solubilize, and removing. The latter approach is desirable at the point in the inside of these that down stream processing is simpler easily [separation of PHA].
[0005] as the approach of obtaining PHA by making biomass constituents other than said PHA solubilizing, and removing -- J.Gen.Microbiology, 19, and 198-209 Page (1958) **** -- the method of processing biomass suspension by the sodium hypochlorite, solubilizing biomass constituents other than PHA, and obtaining PHA is indicated. [for example,] Although this approach is easy as a process, since it is necessary to use the sodium hypochlorite of a large quantity, cost becomes high. Moreover, since the chlorine of an amount which cannot be disregarded in that the remarkable depolymerize of PHA is caused or obtained PHA remains, it is thought that it is not suitable for practical use. Moreover, to JP,4-61638,B, biomass structure is destroyed by heat-treating the microorganism biomass suspension containing PHA above 100 degrees C, subsequently biomass constituents other than PHA are solubilized to it combining protease processing, and phospholipid dialytic ferment processing or hydrogen-peroxide processing, and the method of obtaining PHA is indicated. In order for protein to denaturalize and

insolubilize this approach by heat treatment, down stream processing has faults, such as that the load in the following protease down stream processing increases, and a mostly complicated thing, further.
[0006] moreover, as an approach of having the process which carries out crushing processing of the microorganism biomass to others, after processing with a surfactant, hydrogen-peroxide processing of the nucleic acid emitted from the biomass is carried out, it decomposes, and the method of separating PHA is proposed -- **** (Patent Publication Heisei No. 502415 [eight to] official report) -- in order to use a strong toxic hydrogen peroxide, operation on industrial level is difficult. Moreover, the method of crushing a PHA content microorganism biomass with a high voltage homogenizer, and separating PHA is proposed (JP,7-177894,A). However, as for the purity of PHA which cannot obtain PHA with high purity if this approach repeats microorganism biomass suspension about at least 3 times and high voltage processing is not carried out, and is obtained, the highest also has about 70 - 89%, and the fault of being low.
[0007]
[Problem(s) to be Solved by the Invention] The object of this invention is to offer the separation purification approach of PHA which can obtain PHA of a high grade from the microorganism biomass containing PHA by high yield by the small routing counter.
[0008]
[Means for Solving the Problem] That is, this invention relates to the separation purification approach of PHA characterized by adding a surfactant to the suspension of the microorganism biomass containing a Polly 3-hydroxy alkane acid (PHA), and carrying out physical crushing processing of the mixed liquor obtained.
[0009]
[Embodiment of the Invention] The microorganism used for this invention will not be limited especially if it is the microorganism which is accumulating PHA in intracellular. For example, Alcaligenes RIPORICHIKA (Alcaligenes lipolytica), Alcaligenes, such as Alcaligenes eutrophus (A. eutrophus) and Alcaligenes RATASU (A. latus) (Alcaligenes), Pseudomonas (Pseudomonas), Bacillus (Bacillus), An azotobacter group (Azotobacter), a Nocardia group (Nocardia), The bacillus of Aeromonas (Aeromonas) is mentioned. Especially Strain, such as Aeromonas KYABIE (Aeromonas caviae), Or the strain into which the gene of the PHA synthetic enzyme group of the Aeromonas KYABIE origin was introduced, For example, Alcaligenes eutrophus AC 32 (deposition number FERM P-15786) (J.Bacteriol., 179, and 4821-4830 page (1997)) etc. is more desirable.
[0010] the case where there will be especially no definition if the culture approach of these microorganisms can accumulate PHA into a biomass efficiently so much, for example, said Alcaligenes eutrophus AC 32 (FERM P-15786) is used -- J.Bacteriol., 179, and 4821-4830 Page (1997) etc. -- the approach of a publication is desirable.
[0011] Although the Polly 3-hydroxy alkane acid (PHA) in this invention shows the homopolymer of D-3-hydroxy butyrate (3HB), the copolymer of 3HB and other 3-hydroxy alkane acids, etc. Especially 3HB and D-3-hydroxy hexanoate 2 component copolymer with (3HH) (Macromolecules, 28, and 4822-4828 (1995)) Or 3 component copolymer (JP,8-289797,A) of 3HB, D-3-hydroxyvalerate (3HV), and 3HH(s) It is more desirable from the field of physical properties. Although not limited especially about the presentation ratio of each monomer unit which constitutes 3HB and 2 component copolymer of 3HH(s) here, that whose content of a 1-99-mol % and 3HH unit a 3HB content is 1-99-mol % is suitable. Moreover, although not limited especially about the presentation ratio of each monomer unit which constitutes 3 component copolymer of 3HB, 3HV, and 3HH(s), that whose content of a 1-98-mol % and 3HH unit the content of a 1-95-mol % and 3HV unit is 1-30-mol % for example, for the content of 3HB unit is suitable. Moreover, as for the molecular weight of these PHAs, 100,000 or more are desirable, and 500,000 or more are more desirable.
[0012] the higher one of the content in the microorganism biomass of PHA is desirable -- naturally -- coming out -- it is -- application on industrial level -- setting -- a desiccation bacillus -- when 20 % of the weight or more is inside of the body desirable and the purity of surfactant processing and PHA which physical-crushing-processed, separation-operated and was separated etc. is taken into consideration inside of the body, especially 50 % of the weight or

more is desirable.
[0013] In this invention, a surfactant is added to the suspension of the microorganism biomass which cultivated as mentioned above and was obtained. In addition, "the suspension of a microorganism biomass" in this invention means the water suspension which made water suspend the biomass separated from the culture suspension or the culture medium after culture termination by centrifugal separation etc. The cell mass concentration in this suspension has 500 or less desirable g/l at wet fungus body conversion, and its 300 or less g/l is still more desirable.

[0014] As a surfactant used by this invention, anion nature, cation nature, both sexes, or nonionic are sufficient. Specifically Sodium dodecyl sulfate, dodecyl sulfonic-acid sodium, cholic acid sodium, A sodium deoxycholate, sodium oleate, cetyl trimethylammonium bromide, Dodecyl pyridinium chloride, a 3-(3-cholamidopropyl) (dimethylammonio)-1-propane sulfonic acid, A 3-(cholamidopropyl) (dimethylammonio)-2-HIDORIKISHI-1-propane sulfonic acid, A dodecyl-N-betaine, octyl glucoside, a heptyl thio glucoside, Although poly ethyl ethylene dodecylether, polyoxyethylene iso octyl phenyl ether, the polyoxyethylene nonylphenyl ether, polyoxyethylene sorbitol ester, etc. are mentioned, it is not restricted to those. Especially in this invention, sodium dodecyl sulfate, dodecyl sulfonic-acid sodium, cholic acid sodium, a sodium deoxycholate, sodium oleate, etc. are desirable from the point of a price, the amount used, or the addition effectiveness.
[0015] Although especially the addition of a surfactant is not restricted, its 0.001 - 50 weight section is desirable to the microorganism biomass weight (wet fungus body conversion) 100 weight section, and its 1 - 20 weight section is more desirable. A viewpoint with the good addition effectiveness of a surfactant to more than the 0.001 weight sections of this addition are desirable, and the viewpoint which is low cost to below its 50 weight sections are desirable. Moreover, as for the obtained mixed liquor, it is desirable to stir under a room temperature from a viewpoint which promotes solubilization of biomass constituents other than PHA for 1 minute to about 2 hours.
[0016] Subsequently, physical crushing processing of said mixed liquor is carried out. In this invention, it has the effectiveness of crushing said microorganism biomass and making PHA leaking out of a biomass, by performing starting physical crushing processing.
[0017] With the physical crushing processing in this invention, crushing by crushing by the supersonic wave, the high voltage homogenizer, a mill, etc. is mentioned. As a high voltage homogenizer, APV and the "MANTON gaulin" by the gaulin company of a German country (trade name) Made in [of Denmark] APV Lanny "a mini-laboratory (trade name)", and U.S. micro sieve DIKKUSU (Microfluidica) "Micro sieve TAIZA (trade name)" by the shrine etc. is mentioned. As a mill, it is wheelie A back OFEN (Willy A.Bachofen) of Switzerland. Although "the die no mill (trade name)" by the shrine etc. is mentioned, if the equivalent crushing effectiveness is acquired, it will not be limited to these.
[0018] Although it cannot generally ****** as conditions for physical crushing processing with the means to be used, in crushing by the supersonic wave, it is desirable that it is sonication for 30 minutes at an output 5 and **** cycle 50% made in [U.S.] Branson (Branson) and using a SONIFA year, for example. In crushing by the high voltage homogenizer, the mini-laboratory by APV Lanny of Denmark is used, and it is 500 kgf/cm2. It is desirable that it is high voltage crushing processing of 1 hour. In crushing by a mill etc., it is desirable that it is crushing processing of 1 hour by the flow rate of 1 l/h using the die no mill made from Phi Lea A back OFEN of Switzerland.
[0019] Moreover, said physical crushing processing is completed by checking whether in crushing processing liquid, settlings are obtained, after performing at-long-intervals alignment processing for example, by 3000rpm for a little centrifuging tube for 10 minutes.
[0020] Next, centrifugal separation of the processing liquid obtained by carrying out physical

may be performed two or more times if needed. Moreover, it is desirable to dry the precipitate of obtained PHA with a conventional method.
[0021] The viewpoint of utilization, workability, and physical properties to 90% or more of the purity of obtained PHA is desirable. In addition, as a measuring method of this purity, the approach of a publication is mentioned as the below-mentioned example, for example.
[0022] Moreover, although obtained PHA is a high grade even when it remains as it is, according to the object, it can make peroxides (Patent Publication Heisei No. 502415 [eight to] official report), such as proteolytic enzymes (JP,5-336982,A), such as lytic enzyme (JP,4-61638,B), such as the well-known purification approach, for example, a lysozyme etc., a trypsin, and pronase, and a hydrogen peroxide, etc. able to act, and can raise purity further.
[0023] The purification separation approach of PHA of this invention which has the above configurations has few routing counters compared with the conventional approach, and PHA can be obtained efficiently.
[0024] PHA obtained by this invention has purity high enough as daily necessities, and is suitably used as raw materials, such as fishing implements, such as a plastic, an implant material of recovery needlessness, a drug carrier, fertilizer support, a multifilm for agriculture, and a fishing line, and a garbage bag for compost.
[0025]
[Example] The microorganism used by this example is Alcaligenes eutrophus which introduced the PHA synthetic enzyme group gene of the Aeromonas KYABIE origin. It is AC32 (deposition number FERM P-15786). It is this J.Bacteriol., 179, and 4821-4830 Page (1997) It cultivates by the approach of a publication and is [culture medium. : Na2HPO4.12H2O 11.3g, KH2PO4 1.9g, 2 (NH4) SO4 6g, and pro extract (product made from **** Seasoning) 10g, MgSO4.7H2O 1g, 50g of palm oil, a minute amount metallic element solution (presentation: 3.6H2O16.2 g FeCl) CaCl2.2H2O 10.3g and CoCl2.6H2O 0.2g, NiCl2.6H2O 0.1g, CrCl3.6H2O 0.1g, CuSO4 and 5H2O0.2g/1l, 0.1N HCl 5ml / 1L, pH6.7, the culture temperature of 30 degrees C, culture time amount 72 hour], Pori (D-3-hydroxy butyrate-co-D-3-hydroxy hexanoate) (The following, Pori (3 HB-co-3HH), a 3HB unit:3HH unit = 90:10 (mole ratio) Molecular weight 1 million [about]) The biomass which carried out content about 50% of the weight (dry weight) was obtained. Next, centrifugal separation processing (5000rpm, 10min) separated this from culture medium, water was added to the obtained paste-like biomass, and it considered as the aqueous suspension of 100 g/l (wet fungus body conversion). Although the example shown below was performed using this aqueous suspension, this invention is not limited at all by these examples etc.
[0026] In addition, the purity of Pori (3 HB-co-3HH) obtained from the biomass by dissociating was determined as follows. That is, after dissolving 10mg of dry matters of the precipitate obtained by dissociating from a biomass in chloroform 1ml, methanol 0.85ml and 0.25ml of concentrated sulfuric acid were added, and it processed for 140 minutes at 100 degrees C. After cooling this, it added, and 0.5ml of ammonium sulfate saturated water solutions was stirred violently, they were put, capillary gas chromatography analyzed the lower layer section, and it asked for the purity of Pori in a separation object (3 HB-co-3HH).
[0027] it becomes 1000ml of suspension of the Pori (3 HB-co-3HH) content microorganism biomass of the example 1 above with 10 g/l -- as -- sodium dodecyl sulfate - - in addition, it stirred at the room temperature for 1 hour. Next, fracture processing of this was carried out by the rate of flow of 1 l/h for 1 hour using the "die no mill" (made in [wheelie A back OFEN] Switzerland). Centrifugal separation (8000rpm, 10min) of the obtained processing liquid was carried out, and precipitate was collected. After drying this precipitate, when the purity of Pori (3 HB-co-3HH) was determined, it was 94%.
[0028] it becomes 1000ml of suspension of the Pori (3 HB-co-3HH) content microorganism biomass of the example 2 above with 10 g/l -- as -- sodium dodecyl sulfate -- in addition, it stirred at the room temperature for 1 hour. next, this -- "a mini-laboratory" (Denmark, made in

[0029] Same actuation was performed except having changed sodium dodecyl sulfate into dodecyl sulfonic acid sodium in example 3 example 1. After drying this precipitate, when the purity of Pori (3 HB-co-3HH) was determined, it was 94%.
[0030] Same actuation was performed except having not performed fracture actuation by the "die no mill" in example of comparison 1 example 1. Consequently, even if it carried out centrifugal separation, precipitate could not be obtained, and the polymer was not able to be separated at all.
[0031] Same actuation was performed except having not performed sodium-dodecyl-sulfate processing in example of comparison 2 example 1. Consequently, the purity of the precipitate obtained by carrying out centrifugal separation was the 50 same% as the purity of the Pori (3 HB-co-3HH) content microorganism biomass before suspension.
[0032] After carrying out fracture processing of the 100ml of the suspension of an example of comparison 3 Pori (3 HB-co-3HH) content microorganism biomass by the rate of flow of 1 l/h for 1 hour using a "die no mill", sodium dodecyl sulfate was added and it stirred at the room temperature for 1 hour so that it might become 10 g/l. The obtained biomass suspension was very ****, and even if it carried out centrifugal separation processing, it was not able to obtain Pori (3 HB-co-3HH).
[0033] The above result shows that it is the thing of a high grade compared with what was obtained in the example 2 of a comparison for which no Pori (3 HB-co-3HH) obtained in the examples 1-3 is using the surfactant.
[0034] Moreover, although physical crushing processing and a surfactant need both to be added for the separation purification approach of Pori (3 HB-co-3HH) than the result of examples 1-3 and the examples 1-3 of a comparison, remarkable effectiveness is acquired by carrying out physical crushing processing of the addition processing liquid of a surfactant.
[0035]
[Effect of the Invention] According to this invention, it is efficient, and since it is obtained very simple, this invention contributes greatly the Poly 3-hydroxy alkane acid (PHA) of a high grade to the improvement in effectiveness of industrial production of PHA, and reduction of cost. Moreover, PHA obtained by this invention has purity high enough as daily necessities, and is suitably used as raw materials, such as a fishing implements, such as a plastic, an implant material of recovery needlessness, a drug carrier, fertilizer support, a multifilm for agriculture, and a fishing line, and a garbage bag for compost.

[Translation done.]